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(54) Title: **FAT BASED FOOD PRODUCTS**

(57) Abstract

The invention concerns a fat based food product comprising natural fat components which have a blood cholesterol lowering effect in amounts sufficient to obtain a blood cholesterol lowering effect if the food product is used according to the common needs and customs of the consumer, wherein at least one compound of tocotrienol, oryzanol and phytosterol is present, and preferably at least one compound of oryzanol and phytosterol. In a further preferred embodiment the fat in the product comprises at least 30 wt.%, preferably at least 45 wt.% of pufa-triglycerides. By the regular consumption of the now found fat based food products a positive contribution to health in general, and in particular to the lowering of the blood cholesterol level can be found.

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**Fat based food products**

The present invention concerns a fat based food product comprising natural fat components which have a blood 5 cholesterol lowering effect in amounts sufficient to obtain a blood cholesterol lowering effect if the food product is used according to the common needs of the consumer.

Such fat based food products are available on the market 10 for quite some time, and are to a large extend based on the use of fat which comprises a particular amount of triacylglycerides rich in poly-unsaturated fatty acid (pufa) chains. In particular, the use of butter-like spreads comprising a significant amount of these 15 triacylglycerides having poly- unsaturated fatty acid chains (hereinafter called pufa rich triglycerides) is well known in the western world to reduce high blood cholesterol levels.

20 The variation in the mean plasma total cholesterol concentration among populations is highly correlated with the variation in the extent of atherosclerosis and in the incidence of Coronary Heart Disease (CHD), which is one of the major causes of death in the Western society.  
25 Populations with a low cholesterol level (less than 180 mg/dl (4.7 mmol/L) are found to be less sensitive to atherosclerosis and coronary heart disease, whereas those with mean cholesterol levels above 220 mg/dl (5.7 mmol/L; hypercholesterolaemia) have increased rates of death due to 30 CHD. Thus, a clear need for a method by which the cholesterol level can be lowered exists.

With the common products which comprise triglycerides rich 35 in pufa, a particular amount of fat should be eaten on a daily bases to ensure a high enough intake of these

triglycerides. A contribution of a minimal intake of pufa glycerides of 3 to 4 percent of the metabolizable energy is recommended.

However, if fat based food products are used in which part 5 of the fat is dispensed with, for example by the use of thickeners and/or fat replacers, products with a low fat level such as low fat spreads comprising less than 40% fat would have a less blood cholesterol lowering effect than a product comprising 80% fat, given that a similar amount of 10 pufa rich triglycerides is present in the fat. This is even more the case for the products which were even further reduced in their fat content, such as low fat spreads comprising less than 20% fat.

15 For this type of products, a need for further improvement of the cholesterol lowering effect in fat based food products exists. Also for fat based food products which up till now did not comprise pufa rich triglycerides, the presence of components which have a cholesterol lowering 20 effect is desired.

On the other hand, also the daily consumption of full fat food products per se is decreasing, i.e. not solely by replacement of these products by low fat food products but 25 also by a decreasing consumption of fat based food products as such. This is mainly due to the increasing awareness of the need to reduce the fat intake in general, wrongly based on the scientific consensus towards the necessity to reduce the consumption of triacylglycerides with saturated fatty 30 acids in particular. Because of the reasons mentioned above, for cholesterol reducing PUFA-triglycerides containing fat based products a reasonable consumption is still preferred for the majority of the population in the majority of the effluent countries. Nevertheless, to 35 compensate for the reduction in pufa through reduction of the consumption of triglycerides rich in pufa, also for

those consumers that use high fat food products (e.g. 40-80% yellow fat spreads), which contain more than 30% pufa rich triglycerides on fat, in a decreasing extend, the presence of components that have an additional cholesterol 5 lowering effect is highly preferred.

Also for those consumers for which a significant reduction in plasma cholesterol is desired, i.e. hypercholesterolaemia patients, the consumption of a high 10 or low fat food product that contains more than 30% and preferably more than 45% pufa-triglycerides on fat and also natural components that have an additional cholesterol lowering effect would be most desirable.

15 As consumers these days have a clear preference for food products which are obtained from natural sources our invention deals with food products which contribute to the health of consumers by the use of ingredients obtained 20 from natural vegetable, oil related sources and to include such particular natural ingredients in their context, i.e. in food products comprising ingredients similar to the source of the particular ingredients. In the context of vegetable oil and fat based products, we have now found 25 that some particular natural non-triglyceride minor oil components can be used in food products which are used on a daily basis, and which have a possible contribution to health in general, and in particular to the lowering of blood cholesterol level.

30 In European patent application no. EP 619 952 (Amano), a food additive is described, by which the level of cholesterol in food is lowered by treating the food with the food additive. Upon using the additive, ( $\gamma$ -oryzanol 35 being mentioned), a complex with the cholesterol in the food is formed. The food containing the additive can

therefor not be applied to lower the blood cholesterol level in mammals.

In International Application no. WO 92/19640 (Raison 5 Marganiini oy) a substance of  $\beta$ -sitostanol fatty acid ester is described that can be used as such or added to food. It is described that an ordinary diet contains plant sterols 100-300 mg/day, and that these sterols are poorly absorbed from the intestines. In WO 92/19640, it is thus argued that 10 the use of the plant sterols cannot be used for reducing the serum cholesterol levels in the human diet.

It has now been found that a lowering of blood cholesterol level is obtainable by the regular consumption of fat based 15 food products which comprise at least one compound of the group consisting of tocotrienol, phytosterol and oryzanol, and mixtures thereof, in an amount of at least 0.2 wt% for tocotrienol, 0.25, preferably 0.5 wt%, and further preferred at least 1 wt% for phytosterol and at least 0.25 20 wt%, preferably at least 0.5 wt% and further preferred at least 1 wt% for oryzanol or their relative amounts if mixtures of one or more of these components are used, the weight percentages being based on the total weight of the fat based food product.

25

A preference exists for a fat based food product which comprises a combination of these compounds in amounts. In this embodiment, the fat based food product comprises at least two of tocotrienol, oryzanol and phytosterol, in 30 amounts of at least 0.1 wt% tocotrienol, 0.25 wt% oryzanol and 0.25 wt% phytosterol (to the extent the components are present).

The use of at least one of phytosterol and oryzanol is 35 preferred, these components showing an effect on blood cholesterol even stronger than tocotrienol.

Thus, in another and preferred embodiment of the invention, it has now been found that a significant lowering of blood cholesterol level is obtainable by the regular consumption of fat based food products which comprise at least one compound of the group consisting of phytosterol and oryzanol, and mixtures thereof, in an amount of at least 0.25, preferably 0.5 wt%, and further preferred at least 1 wt% for phytosterol and at least 0.25 wt%, preferably at least 0.5 wt% and further preferred at least 1 wt% for oryzanol or their relative amounts if mixtures of one or more of these components are used, the weight percentages being based on the total weight of the fat based food product.

15 For economical reasons, amounts between 0.5 to 20 wt%, preferably 0.5-10 wt% and further preferred 1-10 wt% for phytosterol, and between 0.25 and 20 wt%, preferably 0.5-20 wt%, more preferred 0.5-10, whereas sometimes an amount between 0.25 and 6 wt% for oryzanol will be sufficient for 20 these ingredients to obtain a cholesterol lowering effect. Of course, higher amounts can be applied if so desired.

In an more preferred embodiment the fat based food product comprises at least 1.2 wt%, preferably at least 4 wt% 25 phytosterol or at least 1.2 wt% preferably at least 4 wt% oryzanol or a mixture thereof in their relative weights. It is preferred in some cases to use more than the relative weights.

Tocotrienol, phytosterol, and oryzanol are hereinafter 30 also referred to as 'healthy components'.

The fat based food products of the present invention comprise preferably more than one compound of the group consisting of 35 tocotrienol, phytosterol, and oryzanol. In this case, the amount of the single components can be less than those

indicated hereinabove, as long as care is taken that for the compound, a relative same weight amount of the other compounds is used. This amount can be indicated as the minimum effective dose (MED). For phytosterol, the minimum 5 effective dose is 0.5 wt%, for oryzanol 0.5 wt%, although sometimes 1 and 1 wt% respectively can be regarded as such. If a lower amount of one or more of these components is used, e.g. 60% of the MED of one of the components is used, a total of 40% of the sum of the MED of the other 10 components should be present as well.

The amounts are amounts normally being sufficient to obtain statistically significant effects after use of these products in amounts similar to the amounts of the prior art 15 fat based products, and for a prolonged period of time.

These amounts are based on common daily intake of the fat based food products. If more or less of such products are being used according to the particular use in a particular 20 area or habits, the amounts can be adapted accordingly.

Tocotrienol, phytosterol, and oryzanol are compounds which are known per se. Japanese patent laid open number 04/320,645 describes the use of unsaponified substances of 25 rice bran oil in rice bran oil, and mentions that the oil exhibited a very good physiological action. No substantiation of this is given.

In the present application, by the use of these components 30 not only a cholesterol lowering effect, but also some antioxidative working can be found.

In a specific embodiment of the present invention, also at least one of tocopherol and polyphenol is present. These 35 components are known as such, and have been described in different publications. For example, in "Tocopherole -

Antioxidantien der Natur; by Pongracz et al.; Fat Sci. Technol.; 97. Jahrgang Nr. 3, pp. 90-104" a study of the use of tocopherols as antioxidants in, inter alia, food is described.

5

Applicants have now found that the use of these healthy minor oil components results in a blood cholesterol lowering stronger than obtainable by the use of any pufa 10 rich triglyceride fat blend alone.

As a further improvement of fat based food products comprising one of these components, it was found that the use of a mixture of these healthy ingredients shows an 15 effect on health, in particular blood cholesterol lowering, which is much stronger than the effect which can be expected on the basis of the studies published regarding the effect of cholesterol lowering per weight amount of intake of each of these components alone. Moreover, this 20 synergy was not found for blood cholesterol lowering alone, but also on other health aspects.

In particular, combinations of oryzanol, phytosterols and pufa rich glycerides show a very beneficial effect which could not be expected on the basis of the health effect of 25 these compounds alone.

The oil related components used in the fat based foods of the present invention can be found as minor components in most plants from which oil is recovered. As conventional 30 oil milling aims at producing triacylglycerols with a minimum amount of unsaponifiable matter, only limited amounts or none of the non-triglyceride healthy components at all are found in the oil presently available. The components to be used in the products of the present 35 invention are found in the waste product streams which

results upon the refining processes for obtaining commonly, classically refined oils.

As only very minor amounts of the suitable components are found in the waste streams of the usual oil refinings,

5 e.g. sunflower oil, soybean oil, rapeseed oil, maize germ oil and the like, using the waste of commonly used seed material will require enormous amounts of the waste material to be further process. However, relative significant amounts of one or more of these components can  
10 be found in materials not always used (yet) on a large scale as an fat or oil source. In particular, the components very suitable for the present invention can be found in, for example, rice bran (in particular oryzanol, phytosterols, and also tocopherols), wheat and maize germ  
15 (in particular phytosterols and tocotrienols), oat and oat bran in particular phytosterols and tocotrienols), sesame seed (in particular phytosterols), soybean, sheanuts (phytosterol) and palm oil (tocotrienols). The healthy minor oil components can also found, although in amounts  
20 lower than the sources mentioned before, in, for example, sunflower, rape, olive, lime, peanuts, cotton, and safflower.

Oryzanol consist of a mixture of ferulic acid esters of  
25 unsaturated triterpene alcohols and is also referred to as gamma-oryzanol. In this invention only the term oryzanol is used. Within the group of tocopherols and tocotrienols one can distinguish alpha, beta, gamma and delta tocopherols and tocotrienols depending on the number and position of  
30 the methyl substituents on the chromane ring of the molecule. In this invention the terms tocopherol and tocotrienol is used to cover this whole family of molecules. For a further description and schematic drawing of the oryzanol and tocopherols and tocotrienols meant in  
35 this description, reference is made to "Separation of Vitamin E and gamma-Oryzanol from Rice Bran by Normal-

Phase Chromatography", M. Diack and M. Saska, JAOCs Vol. 71, no. 11, pp. 1211. Phytosterols, also known as plant sterols or vegetable sterols can be classified in three groups, 4-desmethylsterols, 4-monomethylsterols and 5 4,4'-dimethylsterols. In oils they mainly exists as free sterols and sterol esters of fatty acids although sterol glucosides and acylated sterol glucosides are also present. There are three major phytosterols namely beta-sitosterol, stigmasterol and campesterol. Schematic drawings of the 10 components meant are as given in "Influence of Processing on Sterols of Edible Vegetable Oils", S.P. Kochhar; Prog. Lipid Res. 22: pp. 161-188. In this invention the term phytosterol is used to cover the whole group of free phytosterols, phytosterol fatty acid esters and (acylated) 15 phytosterol glucosides. The term polyphenols (or phenolics) can be defined chemically as a substance which possesses an aromatic ring bearing one or more hydroxy substituents, including functional derivatives. Reference is made to "Phenolic Compounds in Food", Chi-Tang Ho; Phenolic Compounds in Food and Their Effects on Health II, Am. Chemical Soc., 1992. In this invention the term polyphenols refers to all plant phenolic molecules derived from a plant source with an antioxidant activity and not covered by the terms oryzanol, tocotrienol and tocopherol, e.g. simple 20 25 phenols and phenolic acids, hydroxycinnamic acid derivatives (e.g coumaric and ferulic acid) and flavonoids.

Tocopherol and polyphenol can also be obtained from most of these natural vegetable, oil related sources. These 30 components can additionally be present where reference is made to 'healthy components'.

The healthy components can be added as single, pure components, as a mixture of such single, pure components, 35 as components present in a concentrate obtained from specific processing of the natural source, as components

obtained by mixing such concentrates (e.g., obtained from different sources) and the like.

The mixtures of the healthy oil components can be prepared by mixing single, pure components (bought at specialized suppliers) or be obtained by treating the oils and oil containing raw materials in such a way that the minor components remain therein. By specific processing, concentrates comprising a high amount of healthy minor oil components can be obtained. These are applied by preference 10 if the 'naturalness' of a product is considered a particular issue. The concentration of the healthy minor oil components should be such that, if the concentrate is applied, the amounts indicated before are present in the fat based food product. Consequently, this means that the 15 concentration should be high if it is desired to make a product in which the fat is replaced by non-fat ingredients, and a large amount of the other ingredients in the concentrate are fatty components.

20 Fat based food products are food products (partially) based on fat and regarded by the user, in particular the consumer, as 'fatty type of products'. Examples are yellow fat spreads (containing vegetable fat and/or animal fat such as butterfat), dressings, coffeecreamer, shortenings, 25 cooking and frying oils, fillings and toppings, and the like. These products in most cases comprise a particular amount of fat. In some cases, however, products are still regarded as 'fatty type of products', despite a replacement of part or even all the fat by fat replacers. Fat based 30 food products in which the fat is partially or completely replaced by fat replacers are also covered by the term fat based food products of this invention.

Fat products consisting of fat only are also considered as fat based food products in this specification. These are 35 not only used as cooking and frying oils, but also

sometimes in the industry in the preparation of food products, e.g. in baking.

The food products as such are common products in the 5 western world, and are used by consumers on a daily basis in amounts different for each individual.

The invention is in particular very suitable for yellow fat spreads, dressings, cheese, shortenings and cooking and frying oils, and more in particular for yellow fat spreads 10 which can comprise 0 (zero) to 90% fat (usually 5-80%). Dressings can comprise 0 to 85% fat (usually 5-80%), shortenings, cooking and frying oil more than 95% fat.

In a highly preferred embodiment, the food product is a 15 yellow fat spread comprising 0 to 60% fat, preferably 0 to 40% fat, even more preferred 0 to 25% fat, the product comprising at least one of phytosterol and oryzanol and mixtures thereof, in an amount of at least 0.5 wt%, preferably at least 1wt% and more preferred at least 2 wt% 20 for phytosterol, and at least 0.5 wt%, and preferably at least 1 wt% for oryzanol or their relative amounts if mixtures of one or more of these components are used, the weight percentages being based on the total weight of the fat based food product.

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Yellow fat spreads comprising less than 40 wt% fat and at least 1.2 wt% phytosterol and 1.2 wt%, oryzanol can be regarded as very beneficial in that these not only have a very low fat content, but also show an antioxidative effect 30 and a significant lowering of the blood cholesterol working if applied similar to a common butterlike spread on a regular, daily basis. This applies even more for products with less than 25 wt% fat. In another embodiment, the spread comprises at least 3 wt% phytosterol, and optionally 35 also at least 1.2, preferably at least 3 % oryzanol.

The use of the minor healthy components in cheese shows an additional beneficial effect, which is that the cholesterol in cheese is adsorbed by the body in a far lesser amount if phytosterol is present in the cheese than without. Hence,  
5 whereas cheese consumption usually adds cholesterol to the body, with the use of healthy minor oil components, in particular phytosterol, a strongly reduced absorption and a blood cholesterol lowering effect will be found. This effect can also be found for food products other than  
10 cheese comprising cholesterol or consumption of cholesterol containing food products in combination with the products as described in the invention. The amount of minor healthy components to be added on the preparation of the cheese is higher than the amount of cholesterol on a molar weight  
15 basis, so that the cheese product obtained still contains these minor healthy components in a free and active form.

The fat that is applied in these fat based food products can be any fat, such as dairy fat and/or vegetable fat.  
20 However, if fat is present, for health reasons the use of one or more vegetable fat sources is preferred. In particular, the use of liquid fats is preferred. These can be hydrogenated, interesterified, and the like. The fat can be one single fat or a blend. The fat or one of the fats  
25 applied can also beneficially be obtained from the same source as the healthy components are obtained from. E.g., rice bran oil can be obtained and mixed with one or more of the healthy components mentioned in this description which also are obtained from rice bran, or a mixture of wheat  
30 germ oil and rice bran oil can be used with one or more health components obtained from wheat germ and/or rice bran.

The use of fat compositions comprising a considerable  
35 amount of pufa rich triglycerides in addition to the use of the healthy components is in particular considered highly

beneficial. For example, oils of sunflower, safflower, rapeseed, linseed, linola and/or soybean can be used in a preferred embodiment. Also the fat compositions mentioned in Netherlands patent documents no. NL 143115, NL 178559, 5 NL 155436, NL 149687, NL 155177, European patent documents EP 41303, EP 209176, EP 249282, and EP 470658 are highly suitable.

If a fat blend is used, it is preferred that it comprises at least 30%, and more preferred at least 45% of poly-10 unsaturated fatty acids, based on the total weight amount of the fat in the fat based food product. So, a strong effect on the cholesterol lowering effect is obtained if use is made of a mixture of healthy minor oil components such as tocotrienol, phytosterol, and oryzanol, or 15 extracts comprising these components, or these components in combination with the antioxidants polyphenols and tocopherols, or extracts comprising all of these components in a food product in which a fat blend comprising at least 30 wt.% of pufa rich triglycerides is 20 used.

E.g., sunflower oil can be obtained and mixed with one or more of the healthy components mentioned in this description which are obtained from rice bran, wheat germ 25 or a mixture of wheat germ oil and rice bran oil.

In another embodiment of the invention, it has been found that using specific technologies to incorporate the components in the food product significantly increased their physiological beneficial activity. In addition 30 thereto, the solubility of the healthy components in the food product will increase, and, as a result thereof, the maximum concentration can be increased without altering the product characteristics and quality.

35 The techniques which can be applied beneficially are incorporation into the fat by the use of micro-emulsions,

liposomes, incorporation into micro-particles or adhering to micro-particles. According to another method, which is less preferred, one or more of the healthy components that might show a low solubility is esterified as desired with 5 one or more fatty acids to increase their solubility. In the intestine these esters will be hydrolysed resulting in the free fatty acids and active minor oil component. Products so prepared are still considered, for the purpose of this invention, to contain the active minor oil 10 component, as these are hydrolysed in the intestine.

The invention further envisages the use of an oil concentrate comprising more than 4 wt% of one or more of tocopherol oryzanol and phytosterol for the preparation of 15 a fat based food product as described above. In another preferred embodiment, the invention concerns the use of an oil concentrate comprising more than 4 wt% of one or more of tocopherol oryzanol and phytosterol for the preparation of a yellow fat spread as described above.

Example I

Male Syrian hamsters, aged 10-11 weeks, were fed semi-purified diets ad libitum, containing 30% of energy 5 (en%) as fat; 23 en% as proteins; 47 en% as carbohydrates; 0.01% (w/w) cholesterol; and either 0.38% oryzanol as obtained from a commercial source (Tsuno Rice Fine Chemical Ltd, Japan) or 0.34% oryzanol as oil concentrate (using an enriched 4.5% oryzanol containing mixture ( $\pm$  1:12) of rice 10 bran oil and palm oil or nothing (control). The ratio of polyunsaturated (PUFA) to monounsaturated (MUFA) to saturated (SAFA) fatty acids in all the diets was 7.2:12:10.8 (en%). The semipurified diets were fed for 6 weeks, after a run-in period on the control diet for 2 15 weeks. The experimental groups comprised 12-14 hamsters/group. The hamsters were housed individually in Makrolon cages type II, with a layer of sawdust as bedding. At the end of the experiment blood samples were collected 20 and (heparin) plasma total cholesterol was determined using a commercially available enzymic method (CHOD-PAP).

Throughout the experiment body weights and feed intake did not differ between the experimental groups. No clinical signs were observed in any group. The average total plasma 25 cholesterol in the control group was 3.83 mM while the total plasma cholesterol in the group fed with additional pure oryzanol and oryzanol as oil concentrate was on average lowered by 0.57 and 0.71 mM respectively.

30 Example II

Male Syrian hamsters, aged 10-11 weeks, were fed semi-purified diets ad libitum, containing 30% of energy (en%) as fat; 23 en% as proteins; 47 en% as carbohydrates; 35 0.01% (w/w) cholesterol; and either a 100 ppm tocotrienols mixture (composition (%w/w)):  $\alpha$ -tocopherol (20.0),  $\beta$ -

tocopherol (1.1), gamma tocopherol (3.3), delta-tocopherol (0.6),  $\alpha$ -tocotrienol (19.0), gamma-tocotrienol (30.9), delta-tocotrienol (8.0)) or nothing (control). The ratio of polyunsaturated (PUFA) to monounsaturated (MUFA) to 5 saturated (SAFA) fatty acids in the diet was either 7.2:12:10.8 (high PUFA) or 3:12:15 (high SAFA). The semipurified diets were fed for 6 weeks, after a run-in period on the control diet for 2 weeks. The experimental groups comprised 12-14 hamsters/group. The hamsters were 10 housed individually in Makrolon cages type II, with a layer of sawdust as bedding. At the end of the experiment blood samples were collected and (heparin) plasma total cholesterol was determined using a commercially available enzymic method (CHOD-PAP).

15

Throughout the experiment body weights and feed intake did not differ between the experimental groups. No clinical signs were observed in any group, although it was clear that some effect was found which could not be explained 20 without assuming that the tocotrienol did have some kind of effect on the blood cholesterol in the plasma. The average total plasma cholesterol in the control groups on high SAFA and high PUFA diets was 4.25 and 3.95 mM respectively while the average total plasma cholesterol in the groups fed with 25 100 ppm tocotrienol mixture was 4.29 and 4.31 mM for the high SAFA and high PUFA diet respectively, i.e. no cholesterol lowering effect additional to PUFA was observed.

30

Example III

Male Syrian hamsters, aged 10-11 weeks, were fed semi-purified diets ad libitum, containing 30% of energy 35 (en%) as fat; 23 en% as proteins; 47 en% as carbohydrates; 0.01% (w/w) cholesterol; and either oryzanol (using an

enriched 4.5% oryzanol containing preparation dissolved in a mixture ( $\pm$  1:12) of rice bran oil and palm oil (prepared as above)) alone, or in combination with 100 ppm tocotrienols or nothing (control). The ratio of 5 polyunsaturated (PUFA) to monounsaturated (MUFA) to saturated (SAFA) fatty acids in all the diets was either 7.2:12:10.8 (high PUFA) or 3:12:15 (high SAFA). The semipurified diets were fed for 6 weeks, after a run-in period on the control diet for ~2 weeks. The experimental 10 groups comprised 12-14 hamsters/group. The hamsters were housed individually in Makrolon cages type II, with a layer of sawdust as bedding. At the end of the experiment blood samples were collected and (heparin) plasma total cholesterol was determined using a commercially available 15 enzymic method (CHOD-PAP).

Throughout the experiment body weights and feed intake did not differ between the experimental groups. No clinical signs were observed in any group. The average total plasma 20 cholesterol in the high SAFA control groups was 4.25 mM. A decrease on average total plasma cholesterol in the high PUFA control group of 0.30 mM was observed. Addition of oryzanol as oil concentrate decreased the average total plasma cholesterol with 0.60 and 0.47 mM for the high SAFA 25 and high PUFA diets respectively. Additional tocotrienols decreased the average total plasma cholesterol in the high PUFA group further with an additional 0.11 mM. No additional effect of tocotrienols was found in the high SAFA group.

30

The combination of high PUFA and oryzanol as oil concentrate in the diet lead thus to a total decrease of 0.77 mM compared to the high SAFA diet. With additional tocotrienols a total decreasing effect on average plasma 35 cholesterol of 0.88 mM could be found.

Example IV

Male Syrian hamsters, aged 10-11 weeks, were fed semi-purified diets ad libitum, containing 30% of energy 5 (en%) as fat; 23 en% as proteins; 47 en% as carbohydrates; 0.01% (w/w) cholesterol; and either 0.4% phytosterol preparation (ex Kaukas Oy Finland) alone or in combination with 100 ppm tocotrienols or nothing (control). The ratio of polyunsaturated (PUFA) to monounsaturated (MUFA) to 10 saturated (SAFA) fatty acids in all the diets was either 7.2:12:10.8 (high PUFA) or 3:12:15 (high SAFA). The semipurified diets were fed for 6 weeks, after a run-in period on the control diet for 2 weeks. The experimental groups comprised 12-14 hamsters/group. The hamsters were 15 housed individually in Makrolon cages type II, with a layer of sawdust as bedding. At the end of the experiment blood samples were collected and (heparin) plasma total cholesterol was determined using a commercially available enzymic method (CHOD-PAP).

20

Throughout the experiment body weights and feed intake did not differ between the experimental groups. No clinical signs were observed in any group. The average total plasma cholesterol in the high SAFA control groups was 4.25 mM. A 25 decrease on average total plasma cholesterol in the high PUFA control group of 0.30 mM was observed. Addition of phytosterols decreased the average total plasma cholesterol with 0.94 and 0.95 mM for the high SAFA and high PUFA diets respectively. With additional tocotrienols, no additional 30 decreased on the average total plasma cholesterol could be found.

Example V

35 Preparation of a spread 70%

44 parts refined sunflower oil (65% PUFA as linoleic acid) was enriched with oryzanol obtained from rice bran oil and mixed with 18 parts of normal refined sunflower oil and 8 parts of a refined interesterified mixture of 50 parts 5 fully hardened palm oil and 50 parts fully hardened palm kernel oil. To 70 parts of this fatblend, 0.1 part soybean lecithin, 0.1 part monoglyceride and 0.1 part of  $\beta$ -carotene solution are added.

10 To 29 parts water, 0.3 part whey protein powder, a small amount of flavour, and citric acid to obtain a pH of 4.8 are added.

70 parts of the fat phase composition and 30 parts of the 15 aqueous phase composition were mixed and kept at 60°C. The mixture was then passed through a Votator line with 2 scraped surface heat exchangers (A-units) and 1 stirred crystallizer (C-unit) in AAC-sequence operating at 800, 800 and 100 rpm respectively. The product leaving the C-unit 20 had a temperature of 11°C. It was filled into tubs and stored at 5°C. A good and stable, high PUFA, high fat-continuous spread enriched with 2% oryzanol was obtained.

25 Example VI

*Preparation of a spread 40%*

34 parts refined sunflower oil (65% PUFA as linoleic acid) was enriched with oryzanol obtained from rice bran oil and 30 mixed with 6 parts of a refined interesterified mixture of 50 parts fully hardened palm oil and 50 parts fully hardened palm kernel oil. To 39 parts of this fatblend, 0.1 part soybean lecithin, 0.1 part monoglyceride and a small amount of  $\beta$ -carotene solution are added.

To 57 parts water, 2 parts gelatine, 0.3 part whey protein powder, a small amount of flavour, preservative and citric acid to obtain a pH of 4.7 were added.

5 40 parts of the fat phase composition and 60 parts of the aqueous phase composition were mixed and kept at 60°C. The mixture was then passed through a Votator line with 2 scraped surface heat exchangers (A-units) and 2 stirred crystallizers (C-unit), in ACAC-sequence operating at 500,  
10 1000, 600 and 100 rpm respectively. The product leaving the last C-unit had a temperature of 10°C. It was filled into tubs and stored at 5°C. A good and stable, high PUFA, low fat-continuous spread enriched with 1.5% oryzanol was obtained.

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Example VII

*Preparation of a dressing 33% fat*

20 55 parts of water is mixed with 11 parts of various flavour components, preservatives, thickeners and emulsifiers. The mixture is thoroughly mixed in a stainless steel stirred vessel. To this aqueous mixture 33 parts of sunflower oil (65% PUFA as linoleic acid) enriched with oryzanol obtained  
25 from rice bran oil as described in Example 1 and a 1% tocotrienol mixture is added, thoroughly mixed for an additional 15 min, to obtain a pre-emulsion. The pre-emulsion is brought into a colloid mill (Prestomill PM30) and processed at a split-size between level 15 and 20 and a  
30 throughput between level 4 and 6. A good and stable water continuous dressing enriched with 1.5% oryzanol and 1% tocotrienol mixture is obtained.

## Claims:

1. Fat based food product comprising natural fat components which have a blood cholesterol lowering effect in amounts sufficient to obtain a blood cholesterol lowering effect, wherein the fat comprises at least one compound of the group consisting of tocotrienol, phytosterol, and oryzanol in an amount of at least 0.2 wt% for tocotrienol, at least 0.25 wt% for phytosterol, and at least 0.25 wt% for oryzanol, or their relative amounts if mixtures of one or more of these components are used, the weight percentages being based on the total weight of the fat based food product.
2. Fat based food product according to claim 1, wherein the fat comprises at least one compound of the group consisting of phytosterol and oryzanol in an amount of at least 0.5 wt% for phytosterol, and at least 0.5 wt% for oryzanol, or their relative amounts if mixtures are used, the weight percentages being based on the total weight of the fat based food product.
3. Fat based food product according to claim 2, wherein the amounts of the compounds present are in the range of 0.5-20 wt% for phytosterol, 0.5-20 wt% for oryzanol or their relative amounts if mixtures of one or more of these components are used, the weight percentages being based on the total weight of the fat based food product.
4. Fat based food product according to any one of claims 1-3, wherein the food product comprises at least two of tocotrienol, phytosterol, and oryzanol, and preferably in amounts of at least 0.1 wt% of tocotrienol, 0.25 wt% of oryzanol and 0.25 wt% of phytosterol.
5. Fat based food product according to any one of claims 1-4, wherein the fat used in the product is a fat comprising at

least 30 wt%, and preferably at least 45wt% of pufa rich triglycerides, calculated on the total weight of the fat present in the product.

5 6. Yellow fat spread comprising 0 to 60% fat, preferably 0 to 40% fat, the product comprising at least one of phytosterol and oryzanol, in an amount of at least 1 wt% for phytosterol, and at least 1 wt% for oryzanol or their relative amounts if mixtures of one or more of these 10 components are used, the weight percentages being based on the total weight of the fat based food product.

7. Yellow fat spread according to claim 6, wherein the yellow fat spreads comprises less than 25 wt% fat, and at 15 least 1.2 wt% phytosterol and 1.2 wt% oryzanol.

8. Fat based food product according to any one of claims 1-5 or yellow fat spread according to any one of claims 6 to 7, wherein at least one of tocopherol, tocotrienol, 20 phytosterol, oryzanol and polyphenol, is incorporated into the fat.

9. Use of an oil concentrate comprising more than 4 wt% of one or more of oryzanol and phytosterol for the preparation 25 of a fat based food product according to any one of claims 1-5.

10. Use of an oil concentrate comprising more than 4 wt% of one or more of tocopherol, oryzanol and phytosterol for the 30 preparation of a yellow fat spread according to any one of claims 6-7.

## INTERNATIONAL SEARCH REPORT

International Application No  
PCT/EP 96/02344A. CLASSIFICATION OF SUBJECT MATTER  
IPC 6 A23D9/00 A23D7/00 A23L1/24

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)  
IPC 6 A23D A23L A23C C11B

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
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 Further documents are listed in the continuation of box C. Patent family members are listed in annex.

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Date of the actual completion of the international search  3 September 1996	Date of mailing of the international search report  24.09.96
Name and mailing address of the ISA  European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax (+31-70) 340-3016	Authorized officer  Dekeirel, M

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International Application No  
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International Application No  
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